

Remarks

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

A disturbance of amyloid- β homeostasis in Alzheimer's disease leads to the accumulation of this peptide in the form of plaques in the brain. Increased production of amyloid- β peptides or their inadequate clearance can lead to brain accumulation. It has been demonstrated that peptide homologues to amyloid- β form amyloid fibrils in solution if they reach a critical concentration. This process can be effectively promoted by pathological chaperone proteins such as apolipoprotein E, especially its E4 isoform, α 1-antichymotrypsin, or C1q complement factor. They promote formation of amyloid- β fibrils, which remain sequestered within the brain and accumulate in the form of plaques. Inheritance of the apolipoprotein E4 isoform has been identified as a major genetic risk factor for sporadic, late-onset Alzheimer's disease and correlates with an earlier age of onset and greater amyloid- β deposition in an allele-dose-dependent manner. Apolipoprotein E is a 34-kDa glycosylated protein existing in three major isoforms: E2, E3, and E4, which differ in primary sequence at two residues. *In vitro*, all apolipoprotein E isoforms can propagate the β -sheet content of amyloid- β peptides promoting fibril formation, with apolipoprotein E4 being the most efficient. The importance of apolipoprotein E to amyloid- β deposition has also been confirmed *in vivo*. Crossing APP^{V717F} Alzheimer's disease transgenic mice onto an apolipoprotein E knock out background, resulted in a substantial reduction of the amyloid- β load and absence of fibrillar amyloid- β deposits.

Approaches under development for treatment of Alzheimer's disease focus on (1) inhibition of enzymes responsible for amyloid- β cleavage (i.e. amyloid- β secretases), (2) vaccination, and (3) β -sheet breakers (i.e. compound inhibiting amyloid- β fibrillogenesis by directly binding to amyloid- β). Currently, no treatment targeting the pathomechanism of Alzheimer's disease and halting progression of the disease is available.

The present invention is directed to overcoming the deficiencies in existing methods of treating Alzheimer's disease.

The objection to the drawings is respectfully traversed in view of the corrected drawings submitted herewith.

The rejection of claims 1-20 under 35 U.S.C. § 112 (first paragraph) for lack of enablement is respectfully traversed.

In making this rejection, the U.S. Patent and Trademark Office (“PTO”) notes that the specification demonstrates the beneficial effect of amyloid β peptide 12-28P (SEQ ID NO:4) (“A β 12-28P”) to reduce amyloid β plaque formation and increase cell viability. However, it is asserted that the specification does not provide sufficient guidance to enable either a method of administering an agent that inhibits interaction between amyloid β in general and proteins which chaperone amyloid β , or a method involving the treatment or prevention of Alzheimer’s disease.

With respect to the administration of agents that inhibit interaction between amyloid β peptide and proteins which chaperone amyloid β , the PTO’s position is that the specification fails to provide information regarding the nature of such interaction, the degree of inhibition, or a disclosure of the effective conditions for such administration.

As amended, claim 1 is directed to a method of preventing or treating Alzheimer’s disease in a subject, which involves administering to the subject an agent which inhibits interaction between amyloid- β peptide and apolipoprotein E, compared to when the agent is absent, to prevent or treat Alzheimer’s disease in the subject. Amended claim 12 is directed to a method of inhibiting accumulation of amyloid- β peptide deposits in a subject’s brain, which involves administering to the subject an agent which inhibits interaction between amyloid- β peptide and apolipoprotein E, compared to when the agent is absent, to inhibit accumulation of amyloid- β peptide deposits in the subject’s brain. Support for the amendments to claims 1 and 12 is found in the present application, as filed, at original claims 3 and 14, pg. 1, lines 18-21, and pg. 7, lines 30-31.

Applicants submit that the present application provides an enabling disclosure for methods involving the administration of agents that inhibit interaction between amyloid β and apolipoprotein E. In particular, the present application provides a description of amyloid β peptide, apolipoprotein E, and the nature of the interaction between these two proteins (“apoE/A β interaction”), which fully enables a person of ordinary skill in the art to carry out the methods of the present invention. Specifically, apolipoprotein E is a 34-kDa glycosylated protein existing in three major isoforms: E2, E3, and E4, which differ in primary sequence at two residues (present application at pgs. 2-3, ¶ 4). *In vitro*, all apolipoprotein E isoforms can propagate the β -sheet content of the amyloid β peptides promoting fibril formation, with

apolipoprotein E4 being the most efficient (*id.*). The importance of apolipoprotein E to amyloid β deposition has also been confirmed *in vivo* (*id.*). Crossing APP^{V717F} Alzheimer's disease transgenic ("Tg") mice onto an apolipoprotein E knock out background, resulted in a substantial reduction of the amyloid β load and absence of fibrillar amyloid β deposits (*id.*). Apolipoprotein E hydrophobically binds to amyloid β forming SDS insoluble complexes (present application at pgs. 3-5, ¶ 10). Although the affinity of binding depends on amyloid β conformation (amyloid β soluble vs. fibrillar), the binding remains in the low nanomolar range (*id.*). Prior studies identified residues 12-28 of amyloid β as the binding site for apolipoprotein E binding on amyloid β (*id.*). Thus, synthetic peptide homologues to residues 12-28 of amyloid β can be used as competitive agonists of the binding of full length amyloid β to apolipoprotein E (*id.*).

The present application further describes how the peptide agent A β 12-28P effectively blocks apoE/A β interaction *in vivo* and prevents amyloid deposition (pg. 5, lines 5-7). A β 12-28P is just one example of an effective agent for inhibiting apoE/A β interaction. Other agents, such as proteins or peptidomimetics, non-proteinaceous agents, and modified proteins can also be designed and made by well known techniques, as set forth in the present application (pgs. 8-13, ¶¶ 23-45). Identifying agents that inhibit apoE/A β interaction can be accomplished by using e.g., the competitive inhibition assay described in Example 5 of the present application (pgs. 19-20). For example, an inhibition profile of A β 12-28P against apoE/A β interaction is set forth in Figure 3. As further described in Example 15 of the present application, the concentration of an agent producing half-maximal inhibition (IC₅₀) can be calculated from a non-linear regression, one-site competition curve (present application at pgs. 29-30, ¶ 77). In the case of A β 12-28P, the inhibition constant (K_i) was calculated to be 11.37 nmol given the known dissociation constant (K_D) of A β 1-40 binding to apolipoprotein E is approximately 10 nmol (*id.*).

Inhibition of the apoE/A β interaction appears to be nontoxic, because it does not inhibit any physiological reaction (like blocking amyloid β secretase which serves multiple functions) and does not cause an autoimmune response (like the vaccine whose phase II clinical trial was stopped due to morbidity and mortality) (pg. 5, ¶ 11).

In view of the foregoing, the present application not only clearly sets forth an apoE/A β interaction inhibition profile for A β 12-28P and the concentration conditions

effective for achieving reduction in amyloid β plaque depositions but further describes methods for making such determinations with any other inhibition agent.

The present application also teaches effective conditions for administering an agent that inhibits apoE/A β interaction. Specifically, Example 17 at pages 31-32 sets forth administration and treatment of APP/PS1 mice with A β 12-28P, which resulted in reduction of amyloid β load.

Faced with all of this information, a person of ordinary skill in the art would have been fully able to identify and administer agents which inhibit interaction between amyloid- β peptide and apolipoprotein E.

Turning to the issue of preventing or treating Alzheimer's disease, the PTO notes in its outstanding office action that while the administration of the agent A β 12-28P could lead to beneficial reduction of amyloid plaque formation, there is no factual evidence or logical explanation presented to support a conclusion that administration of A β 12-28P (or any other agent) would lead to the treatment of Alzheimer's disease. In support of its position, the PTO cites Sisodia et al., "Role of β -Amyloid Protein in Alzheimer's Disease," *FASEB* 9:366-370 (1995) ("Sisodia"), which states that "[a]lthough necessary, [amyloid β] is not absolutely sufficient for the clinical-pathological features of the disease (i.e., diffuse [amyloid β] deposits occur in normal aged individuals and nonhuman primates, settings in which cognitive changes are not apparent)."

However, applicants submit that the views expressed by Sisodia, published over 11 years ago, do not reflect the current understanding of amyloid β 's role in Alzheimer's disease. As set forth in the present application, accumulation of amyloid β peptide in brains of Alzheimer's disease patients is a hallmark of Alzheimer's disease pathology (pg. 33, lines 1-2). Furthermore, a 2006 review paper describes the role of amyloid β in Alzheimer's disease as follows:

For the last 13 years, the amyloid cascade hypothesis has been the dominant organizing principle behind Alzheimer's research. This hypothesis has held that the initiating molecule in Alzheimer's disease is A β . Over these 13 years, the hypothesis ha[s] been modified in two significant ways: now the plaques are seen as sinks (and perhaps reservoirs) of toxic A β rather than toxic of themselves and the importance of A β 42 as the toxic moiety, rather than total A β has become clearer.

Hardy, "Has the Amyloid Cascade Hypothesis for Alzheimer's Disease been Proved?" *Current Alzheimer Research* 3:71-73 (2006) ("Hardy") (citations omitted) (pg. 71, 1st ¶)

(attached hereto as Exhibit 1). Hardy further states that “[g]iven the neurotoxicity of aggregates of Ab42, the central role of this peptide in AD pathogenesis is self evident” (abstract).

In addition, Sadowski et al., “Blocking the Apolipoprotein E/Amyloid- β Interaction as a Therapeutic Approach for Alzheimer’s Disease,” *PNAS* 103(49):18787-18792 (2006) (“Sadowski”) (attached hereto as Exhibit 2) presents results indicating that compounds that antagonize the apoE/A β interaction constitute an effective therapeutic approach for Alzheimer’s disease (Sadowski at pg. 18787, right col., ¶ 1). Sadowski provides *in vivo* studies in two different Alzheimer’s disease Tg models of mice where A β 12-28P was used to block the apoE/A β interaction (*id.*). Specifically, A β 12-28P was shown to be effective *in vivo* in reducing both the burden of A β deposits and the total A β level in the brains of two Tg model mice (Sadowski at pg. 18791, left col., ¶ 1). In addition to reducing A β parenchymal deposits, treatment with A β 12-28P resulted in a significant reduction in the cerebral amyloid angiopathy burden, which was not associated with any perivascular hemorrhages (*id.*). This observation demonstrates an additional therapeutic benefit of blocking the apoE/A β interaction that has not been observed with immunization against A β (*id.*). Although treatment with A β 12-28P resulted in a decrease in parenchymal and vascular A β deposits along with a decrease in the total A β level, the level of soluble A β and the level of oligomers remained stable (Sadowski at pg. 18791, left col., ¶ 2). Sadowski also reports that unlike mice, human apoE exists in three isoforms (E2, E3, and E4) with single amino acid differences at positions 112 and 158, which have significantly different effects on A β deposition (Sadowski at pg. 18791, left col., ¶ 4). Studies using Tg models expressing human apoE3 or apoE4 isoforms demonstrated that, compared with apoE_{KO} mice, human apoE enhances A β deposition, with E4 producing the strongest effect (*id.*). Therefore, agents blocking the apoE/A β interaction could be applied to human subjects with various apoE backgrounds (*id.*).

For all of these reasons, the lack of enablement rejection of claims 1-20 is improper and should be withdrawn.

The rejection of claims 1-20 under 35 U.S.C. § 112 (second paragraph) for indefiniteness is respectfully traversed in view of the above amendments.

In view of all the foregoing, it is submitted that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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